

Report

Robust Estimation of Experimentwise P Values Applied to a Genome Scan of Multiple Asthma Traits Identifies a New Region of Significant Linkage on Chromosome 20q13

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Over 30 genomic regions show linkage to asthma traits. Six asthma genes have been cloned, but the putative loci in many linked regions have not been identified. To search for asthma susceptibility loci, we performed genome-wide univariate linkage analyses of seven asthma traits, using 202 Australian families ascertained through a twin proband. House-dust mite sensitivity (Dpter) exceeded the empirical threshold for significant linkage at 102 cM on chromosome 20q13, near marker *D20S173* (empirical pointwise $P = .00001$ and genome-wide $P = .005$, both uncorrected for multiple-trait testing). Atopy, bronchial hyperresponsiveness (BHR), and forced expiratory volume in 1 s (FEV₁) were also linked to this region. In addition, 16 regions were linked to at least one trait at the suggestive level, including 12q24, which has consistently shown linkage to asthma traits in other studies. Some regions were expected to be false-positives arising from multiple-trait testing. To address this, we developed a new approach to estimate genome-wide significance that accounts for multiple-trait testing and for correlation between traits and that does not require a Bonferroni correction. With this approach, Dpter remained significantly linked to 20q13 (empirical genome-wide $P = .042$), and airway obstruction remained linked to 12q24 at the suggestive level. Finally, we extended this method to show that the linkage of Dpter, atopy, BHR, FEV₁, asthma, and airway obstruction to chromosome 20q13 is unlikely to be due to chance and may result from a quantitative trait locus in this region that affects several of these traits.

To date, 16 independent samples from 12 countries have been used in genome-wide screens for asthma (MIM 600807) or atopy (MIM 147050) susceptibility loci (table 1). These screens have identified >30 regions of suggestive or significant linkage (Lander and Kruglyak 1995), leading to the positional cloning of four asthma genes or gene complexes: *ADAM33* (MIM 607114) at 20p13 (Van Eerdewegh et al. 2002); *PHF11* (MIM 607796) and *SETDB2* (MIM 607865) at 13q14 (Zhang et al. 2003); *DPP10* (MIM 608209) at 2q14 (Allen et

al. 2003), and *GPRA* (MIM 608595) at 7p14 (Laitinen et al. 2004). Recently, evidence for two new cloned genes at 6p21 (Nicolae et al. 2005) and 5q33 (Noguchi et al. 2005) has been presented. It is likely that some of the remaining linked regions for which a QTL has not yet been characterized will prove spurious. In this respect, replication studies are essential to prioritize regions for subsequent fine mapping.

In this study, we searched for chromosomal regions linked to asthma in a sample of 202 Australian families ascertained through an asthmatic proband. These families were tested as part of a large asthma study performed with the approval of the Human Research Ethics Committee of the Queensland Institute of Medical Research and with the written informed consent of all participants. Detailed methods and phenotype descriptions are provided elsewhere (Ferreira et al., in press). In brief,

Received July 18, 2005; accepted for publication September 8, 2005; electronically published October 14, 2005.

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0002-9297/2005/7706-0017\$15.00

Table 1

Summary of Results from Published Genome Scans of Asthma or Related Traits

REFERENCE(S)	POPULATION(S) ^a	NO. OF FAMILIES (SUBJECTS)	NO. OF MARKERS	POSITION(S) ON CHROMOSOME																
				1	2	3	4	5	6	7	8	9	11	12	13	14	15	16	19	20
Daniels et al. 1996	Australian	80 (364)	296	q35	...	p22	p14	q12
Mathias et al. 2001; Xu et al. 2001a; Blumenthal et al. 2004a, 2004b	CSGA, American	287 (1,931)	323	p32	p13	...
Wjst et al. 1999	German, Swedish	97 (415)	351	p21
Ober et al. 2000	Hutterites, American	1 (693)	563	p21, p36	q34	p22, q12	...	q15	p12, q11, q21	q31	p15	...	q12	q32	q13	...
Yokouchi et al. 2000	Japanese	47 (197)	376	q34	q33	q12
Dizier et al. 2000	EGEA, French	107 (493)	254	q24
Xu et al. 2001b	Chinese	533 (2,551)	422	...	p25	...	q23	p12
Laitinen et al. 2001	Finnish	86 (443)	312	q21	p14
Xu et al. 2000; Koppelman et al. 2002; Meyers et al. 2005; Postma et al. 2005	Dutch	200 (1,174)	366	...	q32	q23-q31	...	q21	q23	q13	...	q12
Haagerup et al. 2002	Danish	48 (206)	446	q24	p24-p22
Hakonarson et al. 2002	Icelandic	175 (1,134)	976	q24
Van Eerdewegh et al. 2002	American, British	460 (920)	387	p13
Yokouchi et al. 2002	Japanese	48 (188)	400	p36	...	p24	...	q33	p13, q24
Bouzigon et al. 2004	French	295 (1,317)	396	p13
Evans et al. 2004	Australian	539 (2,360)	757	...	q33	q24
Kurz et al. 2005	European	82 (366)	603	...	p12	q21	q21	...

NOTE.—Only regions of suggestive ($-\log_{10} P \geq 3.13$ or as demonstrated by simulation) or significant ($-\log_{10} P \geq 4.66$ or as demonstrated by simulation) linkage are shown. Regions of significant linkage are underlined. Results from earlier studies that tested the same phenotypes in the same or a nested sample were not considered.

^a CSGA = Collaborative Study on the Genetics of Asthma; EGEA = Epidemiological Study on the Genetics and Environment of Asthma, Bronchial Hyperresponsiveness, and Atopy.

four affection and three continuous traits were measured in 411 siblings but not in the 180 available parents from these families. The affection traits were self-reported asthma, bronchial hyperresponsiveness (BHR [MIM 600807]), atopy, and house-dust mite (*Dermatophagoides pteronyssinus*) sensitivity (Dpter). A participant was considered to be affected with asthma if he or she answered "Yes" or "Yes, told to me by a doctor" to the question "Have you ever suffered from asthma or wheezing?" and answered "Yes" to the question "Have you ever taken any medicine for asthma or wheezing?" A histamine challenge test was considered to have a positive result if the participant experienced a drop in forced expiratory volume in 1 s (FEV₁) of $\geq 20\%$ of the post-saline inhalation FEV₁ after the last dose of histamine was given. A participant was classified as atopic if at least 1 of 11 common allergens tested elicited a mean wheal diameter at least 3 mm greater than the negative control wheal. Similarly, a skin-prick test for Dpter was considered to have positive a result if the mean wheal diameter was 3 mm greater than the negative control wheal. The three continuous traits were baseline FEV₁; baseline airway obstruction, as measured by FEV₁ divided by forced vital capacity (FEV₁/FVC); and total serum immunoglobulin E (IgE) levels. These were all measured in accordance with standard procedures (Ferreira et al., in press) and were normalized prior to analysis.

The characteristics of the 591 participants used for this genome scan are summarized in table 2. We have shown elsewhere that the seven traits of interest have a high reliability (test-retest correlation range 0.67–0.99), are all heritable (heritability range 0.38–0.71), have cross-trait phenotypic correlations across a wide range (0.03–0.81; mean 0.35), and are regulated to a great extent by specific genetic factors (Ferreira et al., in press). Nonetheless, there was some degree of genetic overlap, particularly between atopy and both asthma and BHR and, as expected, between markers of allergic sensitization (atopy, Dpter, and IgE).

In addition to these phenotypes, genomewide genotyping data were obtained for the 202 families as part of four recent twin studies of asthma (Ferreira et al., in press), neuroticism (Kirk et al. 2000), cardiovascular disease (Beekman et al. 2003), and other common disorders (Whitfield et al. 2000). When data from these four sources were combined, the genome-scan sample consisted of 87 families (43%) with no parents genotyped, 50 (25%) with one parent genotyped, and 65 (32%) with both parents genotyped. Of 218 sib pairs available for analysis, 14 (6%) were genotyped at 201–300 markers, 104 (48%) at 301–500 markers, 54 (25%) at 501–1,000 markers, and 46 (21%) at 1,001–1,544 markers. Familial relationships were verified using GRR (Abecasis et al. 2001), Mendelian inconsistencies were identi-

Table 2

Characteristics of 591 Participants from 202 Families Used for a Genome Scan of Multiple Asthma Traits

Characteristics	Parents	Twins and Sibs
Subjects:		
Total	180	411
With questionnaire	7%	100%
With full testing	0%	99%
Female sex	59%	57%
Mean (range) age [years]	63 (45–80)	38 (22–75)
Ever a smoker	46%	50%
Current smoker	0%	26%
Asthma	23%	38%
Doctor-diagnosed asthma	23%	31%
Positive BHR test	...	48%
Atopy	...	73%
Positive SPT for <i>D. pteronyssinus</i> ^a	...	58%
FEV ₁ \leq 0.7 predicted	...	6%
Mean (range) FEV ₁	...	3.3 (.7–5.7)
Mean (range) FEV ₁ /FVC8 (.4–1.0)
IgE geometric mean (range) [IU/ml]	...	71 (.6–6,908)

^a SPT = skin-prick test.

fied and removed using SIB-PAIR 0.99.9 (see Web Resources), and unlikely recombinants were identified and removed using MERLIN 1.0 (Abecasis and Wigginton 2005). The marker genetic positions were interpolated via locally weighted linear regression from the National Center for Biotechnology Information (NCBI) build 34.3 physical map positions and the published Rutgers genetic map (Kong et al. 2004), and they are expressed in Kosambi cM (see genetic map site in Web Resources). The average multipoint entropy information content (Kruglyak et al. 1996) was 0.57 (range 0.15–0.85), computed with MERLIN at the marker closest to the middle of the chromosome and averaged over the 22 autosomes. The average heterozygosity for the autosomal markers was 75%. The 218 available sib pairs were genotyped, on average, at 624 autosomal markers (range 201–1,544). Intermarker distances were estimated for each sib pair and then were averaged across the 218 pairs. The mean intermarker distance was 7.1 cM (range 2.8–15.8).

Two sets of univariate linkage analyses were performed. For both sets, the probabilities that two siblings shared 0, 1, or 2 alleles identical by descent (IBD) were estimated every 2 cM with MERLIN. For the four affection traits, the Whittemore and Halpern (1994) S_{all} statistic was calculated, and Z scores were converted into LOD scores by use of the Kong and Cox (1997) exponential model implemented in MERLIN. This analysis is appropriate for the type of sample-selection procedure used. For the three continuous traits, IBD probabilities were imported into SOLAR 2.1.4, and maximum-likelihood univariate variance-components linkage analysis was performed as implemented by Almasy and Blangero

(1998). In addition, the fixed effects of significant ($P < .05$) covariates—age, sex, height (for FEV₁ only), and regular smoking (for FEV₁/FVC only)—were included in the means model. We have shown elsewhere that the ascertainment procedure had minimal impact on variance-components estimates of FEV₁, FEV₁/FVC, and IgE (Ferreira et al., in press), which reflects the low phenotypic correlations between these traits and asthma (range 0.25–0.29). Nonetheless, we used the proband-ascertainment correction implemented in SOLAR for variance-components linkage analyses. Residual kurtosis was 1.5 for FEV₁, 0.43 for FEV₁/FVC, and –0.36 for IgE.

Since the distribution of linkage test statistics was not comparable between affection and continuous traits or even between different continuous traits, all LOD scores were converted to empirical pointwise P values. In brief, to assess pointwise significance, we used MERLIN to generate 1,000 genome-scan replicates that retained the original phenotypes but had new genotypes simulated under the null hypothesis of no linkage for all autosomes, preserving the same allele frequencies, missing data pattern, and marker spacing. Linkage analysis was then performed for each trait with each genome-scan replicate, as described for the real data set. LOD scores observed with these simulated data sets were entirely due to chance. Once each trait was analyzed with the real genome scan and with the 1,000 genome-scan replicates, all LOD scores for that trait were combined into a single distribution. The empirical pointwise P value for each LOD l , obtained with the real or simulated data sets, was then computed as the proportion of LOD scores in this distribution that were $\geq l$. All empirical pointwise P values are conveniently expressed as the $-\log_{10}$ of the P value ($-\log_{10}P$).

To assess genomewide significance, we further investigated how often a given $-\log_{10}P$ was expected to be observed in a genome scan by chance alone. This was performed for each of the seven traits individually. Briefly, for each of the genome-scan replicates described above, we recorded the highest empirical pointwise $-\log_{10}P$ observed for each chromosome and counted the number of chromosomes exhibiting a $-\log_{10}P$ equal to or greater than a given threshold j . The empirical genomewide thresholds for suggestive or significant linkage (Lander and Kruglyak 1995) were defined as the threshold j for which we observed, on average, 1 or 0.05 peaks per genome scan with a $-\log_{10}P \geq j$, respectively. For all traits, a $-\log_{10}P$ of 2.1 was the threshold for suggestive linkage, and a $-\log_{10}P$ of 4.0 was the threshold for significant linkage.

The strongest evidence of linkage in the univariate analyses occurred at 96–102 cM on chromosome 20q13, near markers *D20S171* and *D20S173* (fig. 1A–1G). At this location, significant linkage was observed for Dpter

(empirical pointwise $-\log_{10}P = 4.93$), and suggestive linkage was observed for atopy ($-\log_{10}P = 3.21$), BHR ($-\log_{10}P = 2.64$), and FEV₁ ($-\log_{10}P = 2.45$). The mean proportion of alleles shared IBD at 20q13 was 0.62 (95% CI 0.55–0.69) for sib pairs concordant affected for Dpter, 0.52 (95% CI 0.46–0.58) for discordant pairs, and 0.49 (95% CI 0.39–0.58) for concordant unaffected pairs. Similar patterns of allele sharing were observed for atopy and BHR. The proportion of variance in FEV₁ explained by a QTL in this region was 0.50 (95% CI 0.21–0.79), but this figure should be interpreted with care because effect sizes estimated from genomewide scans are known to have a large upward bias (Goring et al. 2001). Sixteen additional regions exceeded the threshold of suggestive linkage for at least one of the seven traits (table 3).

Some of the regions identified with univariate analyses were expected to be false positives arising from multiple-trait testing. The first approach used to address this problem was to perform variance-components analysis of multiple asthma traits by use of the algorithm implemented in SOLAR 3.0.3 and outlined by Williams et al. (1999b). Multivariate linkage analyses of the three continuous traits (FEV₁, FEV₁/FVC, and IgE) improved the evidence of linkage at 20q13 and 12q24, compared with the individual univariate analyses of these three traits (not shown). However, linkage models could not be consistently maximized when affection traits were added to the multivariate analysis of the three continuous traits. In addition, estimation of empirical significance was not feasible, with one genome-scan replicate of three traits taking ~2 wk to run on a 2.80-GHz processor. As a result, multivariate analysis could not be used to overcome the problem of multiple-trait testing.

We then developed and applied a simple approach to estimate genomewide significance while accounting for multiple-trait testing and for the correlation structure between traits. To exemplify our approach, consider the empirical pointwise $-\log_{10}P$ observed for the seven traits at position 102 cM on chromosome 20: 1.04 (asthma), 2.59 (BHR), 3.21 (atopy), 4.93 (Dpter), 2.45 (FEV₁), 0.95 (FEV₁/FVC), and 0.30 (IgE). Note that these $-\log_{10}P$ refer specifically to position 102 cM, whereas table 3 reports the highest $-\log_{10}P$ for each trait within the 96–102 cM range. First, we sorted these $-\log_{10}P$ values in descending order to create the observed vector of ordered $-\log_{10}P$ values, $V_Q = [4.93, 3.21, 2.59, 2.45, 1.04, 0.95, 0.30]$. This same procedure was used to create one simulated vector (V_{Null}) for each position of the 1,000 genome-scan replicates simulated under the null hypothesis of no linkage (see above). We analyzed 1,796 positions throughout the 22 autosomes (3,548 cM at 2-cM intervals), and thus 1,796 V_{Null} vectors of ordered $-\log_{10}P$ values were created for each genome-scan replicate. Note—and this is crucial—that the order of traits

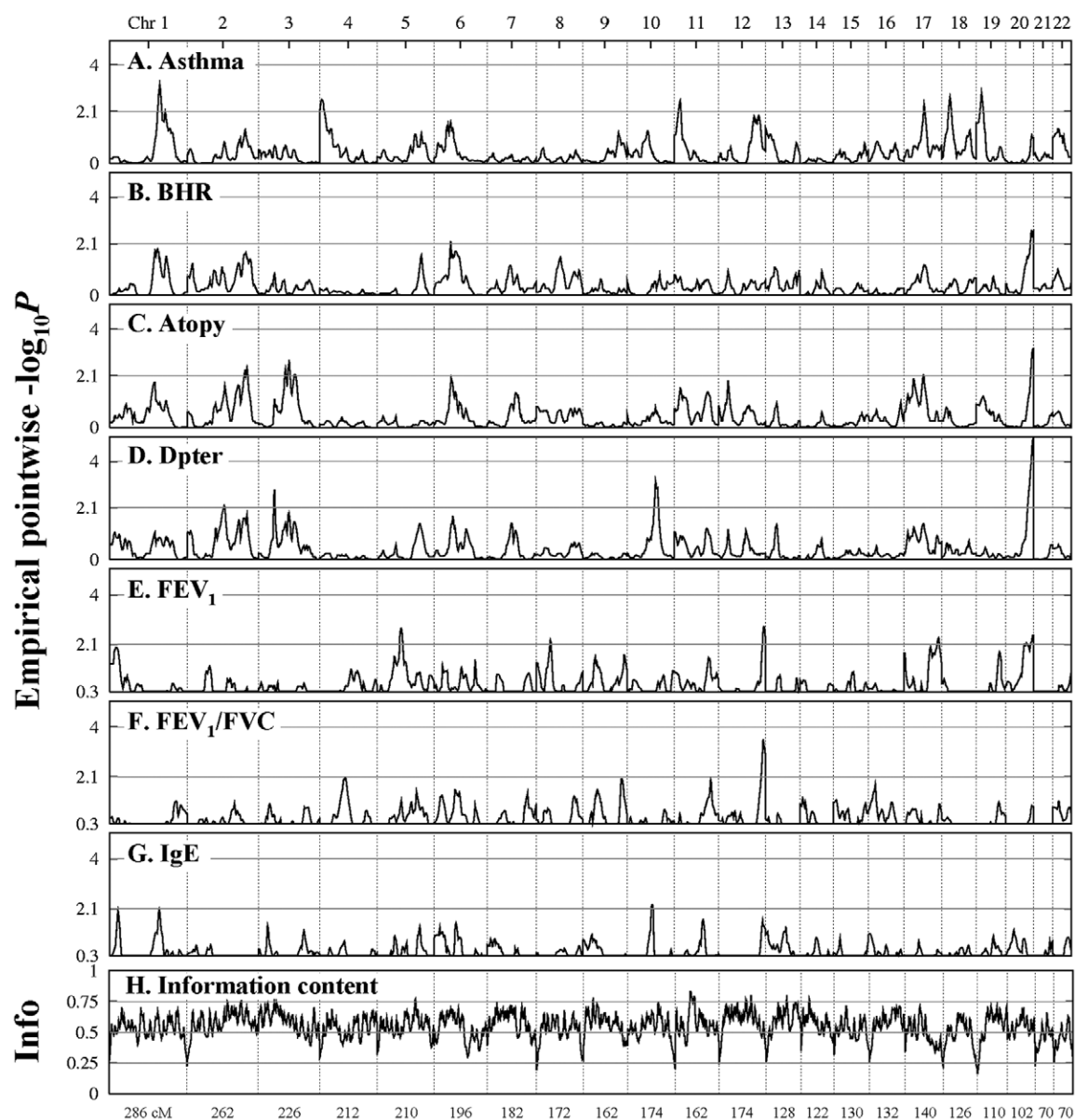


Figure 1 Genomewide linkage results for asthma and related traits. Autosomes are arranged by number from p-ter to q-ter, with genetic distance expressed as Kosambi cM (0–3,548 cM). A–D, For the four affection traits, the Whittemore and Halpern (1994) S_{all} statistic was calculated, and Z scores were converted into LOD scores by use of the Kong and Cox (1997) exponential model implemented in MERLIN 1.0. LOD scores were then converted to empirical pointwise $-\log_{10}P$ values by use of 1,000 genome-scan replicates simulated under the null hypothesis of no linkage. The same replicates were used to estimate the thresholds for genomewide suggestive and significant linkage (see main text). The number of families with two or more affected sibs was 34 for asthma, 49 for BHR, 115 for atopy, and 69 for Dppter. E–G, For the three continuous traits, IBD probabilities were imported into SOLAR, and maximum-likelihood univariate variance-components linkage analysis was performed with the fixed effects of covariates (see main text) and proband-ascertainment correction. LOD scores were converted to empirical pointwise $-\log_{10}P$ values, and genomewide thresholds were estimated as described above. The minimum value for the Y-axis corresponds to $-\log_{10}(.5)$ (i.e., LOD = 0). The top horizontal line shows the empirical genomewide threshold for significant linkage ($-\log_{10}P = 4$, for all traits), and the bottom line, for suggestive linkage ($-\log_{10}P = 2.1$). H, Multipoint entropy information content (Kruglyak et al. 1996). The average information content was 0.57, computed with MERLIN at the marker closest to the middle of the chromosome and averaged over the 22 autosomes.

Table 3**Univariate Linkage Results with $-\log_{10}P \geq 2$**

REGION	LOCATION ^a (cM)	PEAK MARKER(S)	EMPIRICAL POINTWISE $-\log_{10}P$ FOR ^b						
			Asthma	BHR	Atopy	Dpter	FEV ₁	FEV ₁ /FVC	IgE
1q24-q25	182–184	<i>D1S196, D1S1589</i>	3.20	2.08
2q14	136	<i>D2S347</i>	2.19
2q35	220	<i>D2S434</i>	2.43
3p23	60	<i>D3S1768</i>	2.86
3q12	114	<i>D3S1271</i>	2.75
4p16	8	<i>D4S412</i>	2.60
4q21	94	<i>D4S400</i>	2.07	...
5q13	90	<i>D5S424</i>	2.71
6p21	62–64	<i>D6S426, D6S1017</i>	...	2.20	2.01
8p21	52	<i>D8S1771</i>	2.26
9q34	142	<i>D9S290</i>	2.02	...
10q22	92–104	<i>D10S535, D10S1686</i>	3.20	2.26
11p15	22	<i>D11S1999</i>	2.55
12q24	166–168	<i>D12S343, D12S1723</i>	2.78	3.53	...
17q21	72–74	<i>D17S791</i>	2.41	...	2.13
17q25	128	<i>D17S784</i>	2.35
18p11	30	<i>D18S843</i>	2.70
19p13	20	<i>D19S1034</i>	2.87
20q13	96–102	<i>D20S171, D20S173</i>	...	2.64	3.21	4.93	2.45

^a Marker locations are given in Kosambi cM and have been interpolated from published physical positions and genetic maps (Kong et al. 2004).

^b Empirical pointwise P values and genomewide thresholds were estimated for each trait from 1,000 genome-scan replicates generated under the null hypothesis of no linkage (see main text for details). Empirical genomewide thresholds for all traits were 2.1 for suggestive linkage and 4.0 for significant linkage.

does not have to be the same in the observed vector V_Q and in the simulated vectors V_{Null} , a property that forms the basis for correction of multiple-trait testing. Second, to compute the genomewide P value for trait k of V_Q , we counted the proportion of genome-scan replicates that had at least one V_{Null} vector with a highest $-\log_{10}P$ greater than or equal to the $-\log_{10}P$ recorded for trait k of V_Q .

When this approach was applied to Dpter at 20q13, 42 of 1,000 genome-scan replicates had one or more V_{Null} vectors with a highest $-\log_{10}P \geq 4.93$. Therefore, the genomewide P value for Dpter at this position remained significant after multiple-trait testing was correctly accounted for ($P = .042$). Note that, if we had applied the traditional approach of estimating the genomewide significance for Dpter individually, then P would be equal to .005—that is, there were 5/1,000 Dpter genome-scan replicates with one or more positions that exceeded a $-\log_{10}P$ of 4.93. If a Bonferroni correction were then used to account for multiple-trait testing, the genomewide P would lie between .025 (correction for five independent traits, as identified by principal-components analysis) and .035 (correction for seven traits tested). Of the remaining 16 regions of suggestive linkage shown in table 3, only the linkage between 12q24 and FEV₁/FVC was at the suggestive level after multiple-trait testing was accounted for, with an

expected number of false positives per genome scan of 0.741 ($P = .509$).

Six traits were linked to chromosome 20q13, although only Dpter showed linkage at the significant level (fig. 2). Next, we investigated whether the simultaneous linkage of multiple traits to 20q13 could be attributed either to a pleiotropic QTL in this region or simply to chance. For this purpose, we extended the approach described above to estimate the probability that a genome scan simulated under the null hypothesis of no linkage could have one or more V_{Null} vectors with the sum of the k highest $-\log_{10}P$ values (i.e., $S_k = \sum_{i=1}^k -\log_{10}P_i$) greater than or equal to the sum of the k highest $-\log_{10}P$ values of V_Q at 102 cM on 20q13, where $k = 1, 2, \dots, 7$ (table 4). A similar method was recently proposed to combine P values obtained in performing association analysis of multiple SNPs, such as in genomewide association studies (Hoh et al. 2001; Dudbridge and Koeleman 2004). The significance of the S_k statistics computed at V_Q was expected to decrease as k approached the number of traits influenced by an underlying QTL and to stabilize thereafter (for similar examples, see Hoh et al. [2001] and Dudbridge and Koeleman [2004]). Note that the same seven traits were analyzed with the real genome scan and with each genome-scan replicate, which thus preserved the original correlation structure between traits in the analyses performed under the null hypothesis.

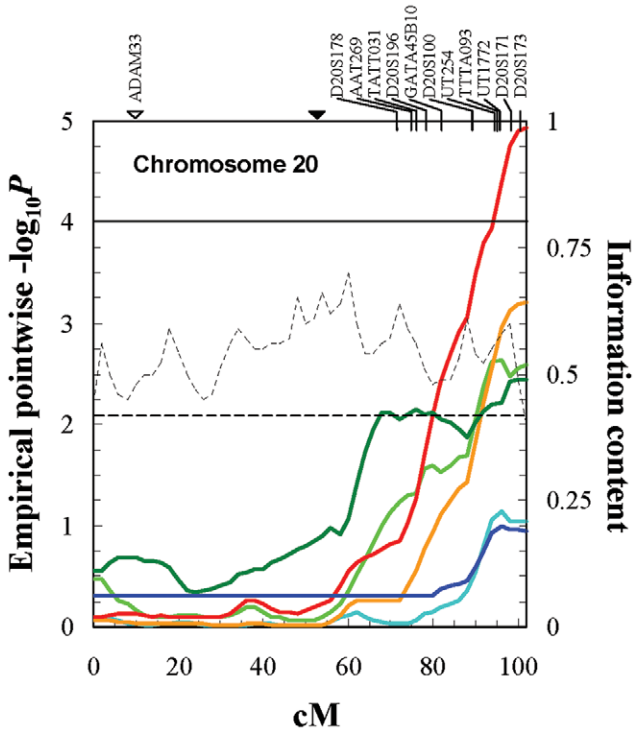


Figure 2 Details of linkage results on chromosome 20 for Dpter (red), atopy (orange), BHR (light green), FEV₁ (dark green), asthma (light blue), and FEV₁/FVC (dark blue). The solid horizontal line shows the empirical genome-wide threshold for significant linkage, and the dashed line shows the threshold for suggestive linkage (see main text). Multipoint entropy information content (Kruglyak et al. 1996) is also shown (dotted line). Highlighted at the top are the positions of ADAM33 (unblackened arrowhead), the centromere (blackened arrowhead), and the markers genotyped in the 20q terminal region. Note that the X-axis scale ends at 102 cM, although the last X-axis label is 100 cM for convenience.

When only the highest $-\log_{10}P$ at 20q13 was considered (4.93 for Dpter), the genome-wide P value was .042, as described above. However, when we considered the cumulative $-\log_{10}P$ observed for Dpter and atopy (8.14), only 26/1,000 genome-scan replicates had at least one simulated vector with the sum of the two highest $-\log_{10}P \geq 8.14$ ($P = .026$). If the linkage of atopy to 20q13 was spurious or a result of the residual correlation between this trait and Dpter, then no improvement in significance would be expected when both traits were considered. Similarly, only 7/1,000 genome-scan replicates had one or more simulated vectors with a sum of the six highest $-\log_{10}P$ values ≥ 15.17 ($P = .007$). Even the addition of the FEV₁/FVC trait, which had an empirical pointwise $-\log_{10}P$ of only 0.95, considerably increased the evidence for linkage in this region. On the other hand, taking the IgE trait into consideration had no impact on the overall linkage significance, which sug-

gests that a QTL in this region either does not regulate variation in total serum IgE levels or does so only to a degree that is undetectable in this sample. Therefore, our results suggest that the simultaneous linkage of Dpter, atopy, BHR, FEV₁, asthma, and FEV₁/FVC to chromosome 20q13 is unlikely to be due to chance or to the residual correlation between these traits and may result from a pleiotropic or multiple QTL that map(s) to this region.

Finally, since we computed seven S_k sum statistics in this process, we estimated the global genomewide significance of linkage between the six traits and 20q13, using a permutation test similar to that proposed by Hoh et al. (2001). (Note: The SAS routines used to estimate genomewide significance in the present study are available from the authors upon request.) In this case, the test consisted of counting the proportion of genome-scan replicates that had one or more V_{Null} vectors with any of the seven S_k sum statistics as significant as the S_k statistic observed at 20q13 when $k = 6$ (with $P = .007$). We observed 23 of such genome-scan replicates among the 1,000 total replicates, and thus the global genomewide significance for multiple linkage at 20q13 was $P = .023$. Although it was beyond the scope of this study, we performed simulations that showed that this method of combining results from univariate linkage analyses can be as powerful as formal multivariate variance-components analysis in detecting a pleiotropic QTL, with the advantage that it is computationally feasible for any number of traits, even if these are analyzed with different linkage statistics (not shown).

In this study, we searched for chromosomal regions linked to asthma traits measured in 202 Australian families ascertained through a twin proband. Univariate analyses of seven traits identified 1 region of significant

Table 4
Empirical Genomewide Significance of the Seven S_k Sum Statistics Recorded at Position 102 cM on Chromosome 20q13

Rank (k)	Trait	$-\log_{10}P^a$	Cumulative $-\log_{10}P (S_k)$	P^b
1	Dpter	4.93	4.93	.042
2	Atopy	3.21	8.14	.026
3	BHR	2.59	10.73	.017
4	FEV ₁	2.45	13.18	.012
5	Asthma	1.04	14.22	.011
6	FEV ₁ /FVC	.95	15.17	.007
7	IgE	.30	15.47	.007

^a Empirical pointwise $-\log_{10}P$ for each trait at 102 cM on 20q13.
^b Empirical genomewide significance of S_k . Corresponds to the proportion of genome-scan replicates with one or more positions with an S_k statistic greater than or equal to the S_k observed at 20q13 in the real scan. The probabilities were estimated from 1,000 genome-scan replicates simulated under the null hypothesis of no linkage (see main text).

linkage and 16 of suggestive linkage. Some of these regions have previously been identified by more than one study at a suggestive or significant level, in particular 2q33-q35, 6p21, and 12q24 (table 1). However, it was likely that some were, in fact, false-positives arising from multiple-trait testing. The most common approach to address this issue is to perform multiple univariate analyses and then apply a Bonferroni correction to the observed *P* values. This approach, however, has well-known limitations. First, the significance of a particular position (i.e., marker) is assessed separately for each trait, which can result in many traits being linked to the same position but none at a particularly significant level. Second, the Bonferroni correction can be conservative in some situations and, thus, may contribute to a reduction in the power of the analysis.

One alternative is to perform a principal-components analysis of all traits tested and then analyze only the extracted independent factor scores. This approach has been applied previously to asthma (Holberg et al. 2001). However, although it overcomes the problem of testing correlated traits, this approach minimizes but does not entirely overcome the problem of testing multiple traits, and it may result in testing of phenotypes that are not comparable to those in other studies. Another alternative is to perform a full multivariate analysis of all phenotypes tested. This was recently applied to traits such as alcoholism (Williams et al. 1999a), dyslexia (Marlow et al. 2003), and metabolic syndrome (Olswold and de Andrade 2003). Although theoretically desirable, the multivariate approach also has some limitations. First, multivariate linkage is not always guaranteed to provide increased power compared with that of individual univariate analyses. Martin et al. (1997) showed that power is improved if the pattern of factor loadings in the QTL is correctly specified but is reduced if it is incorrectly specified. Furthermore, the gain in power seems to be considerable only when a pleiotropic QTL and the residual sources of variation induce cross-trait correlations in opposite directions (Allison et al. 1998; Amos et al. 2001; Evans and Duffy 2004). An additional limitation of multivariate analysis is computational. The two methods available for multivariate linkage are the variance-components method (Eaves et al. 1996; Blangero and Almasy 1997; de Andrade et al. 1997) and a modified Haseman-Elston method (Amos et al. 1990). In theory, variance components can be extended to any number of traits, but, in practice, only a restricted number of traits can be effectively analyzed with current tools, particularly if both continuous and affection traits are being measured or if empirical significance will be estimated. This was the case for the multivariate analyses reported here. Continuous and affection traits could not be analyzed simultaneously, and empirical significance could not be assessed. Similarly, at present, the multivariate

Haseman-Elston method is limited to sib pairs and provides lower power than does the multivariate variance-components method (Amos et al. 2001).

Thus, commonly used approaches to address multiple-trait testing in the context of linkage analyses are limited in their power and/or applicability. We sought to develop a general method for the assessment of experimentwise linkage significance that took into account the information provided by the multiple univariate analyses performed. The approach outlined and implemented here efficiently overcomes the acute problem of multiple-trait testing without resorting to a Bonferroni correction. In the first published genomewide screen for asthma, Daniels et al. (1996) used a permutation method that is similar in principle to our approach; unfortunately, very few genomewide linkage studies adopted their method. In addition, the approach we describe here can be extended to assess the evidence for linkage at a particular position across a number of traits. When this method was applied to our genome scan of seven asthma traits, two regions were identified that exceeded the genomewide threshold for suggestive (12q24) or significant (20q13) linkage. Chromosome 12q24 was linked to the lung function traits FEV₁/FVC and FEV₁. To date, three studies have reported suggestive or significant linkage to 12q23-q24 (table 1), but a major asthma gene has yet to be cloned in this region. A number of association studies suggest that the nitric oxide synthase 1 gene (*NOS1* [MIM 163731]) is a likely candidate (Grasemann et al. 1999; Gao et al. 2000; Wechsler et al. 2000; Ali et al. 2003; Shao et al. 2004).

However, the most significant evidence for linkage was observed at a novel region, chromosome 20q13. Multiple traits were linked to this region, including Dp_{ter}, atopy, BHR, FEV₁, asthma, and FEV₁/FVC. The simultaneous linkage of these six traits to 20q13 was expected by chance alone in only 7 of 1,000 genome scans. Therefore, we suggest that a pleiotropic QTL and/or multiple clustered QTL in this region contribute(s) to the variation of Dp_{ter}, atopy, BHR, and FEV₁, although to different degrees. A QTL at 20q13 may also directly influence asthma status and variation in FEV₁/FVC. To our knowledge, no study has reported suggestive or significant linkage of asthma traits to this region. There are many examples of genes shown to be associated with asthma for which suggestive or significant linkage was reported in only a small number of independent samples, including *ADAM33* and *DPP10*. This may result from a combination of factors—namely, genetic heterogeneity and, in the case of loci located near terminal positions, low inheritance information available toward the telomeres. A number of chromosomes in our genome scan displayed such effects, but the entropy information content at the last 10 cM of chromosome 20—where our six peaks lie—ranged from 0.40 to 0.60, which is com-

parable to the genomewide mean (0.57). Singlepoint analyses provided further confidence that these results are unlikely to be driven by only one marker or to be an artifact caused by end-of-map problems. Dp11 showed singlepoint LOD scores ≥ 1.0 for *D20S100*, *UT254*, *TTTA093*, and *D20S171* and ≥ 0.7 for *AAT269*, *GATA45B10*, *UT1772*, and *D20S173* (see fig. 2 for positions).

Finally, the 20q13 linkage region is broad, with the equivalent of a 1-LOD drop interval of 12 cM (8.7 Mb) situated between *D20S100* and 20qter. At present, there are 117 known genes in this segment, of which only the cadherin-like 26 gene (*CDH26*) stands out as a candidate gene for atopy or asthma. Although *ADAM33* is also located on chromosome 20, it is 54.7 Mb distant and, thus, is very unlikely to explain our linkage signal. However, we note that most, if not all, cloned asthma genes had no obvious functional relevance when first identified. Therefore, subsequent fine-mapping strategies are warranted across the entire 20q13 linkage region.

Acknowledgments

We thank all the families who participated in this study. We also thank Dr. Claire Wainwright, Barbara Haddon, Dixie Statham, Wendy Oddy, Sunalene Devadason, Dr. Nigel Dore, Marita Dalton, Brigitte Willemsse, Elena Belousova, Janet Li, and Dr. Jennifer Peat, for phenotype collection; Dr. Jeff Hall (Sequana scan), Dr. Peter Reed (Gemini Genomics scan), Dr. Eline Slagboom, Dr. Bas Heijmans, and Professor Dorret Boomsma (Leiden scan), and Dr. James Weber (Marshfield scan), for genotype collection; Sarah Medland and Kate Morley, for their work with some of the genotyping data; Gu Zhu, David Smyth, and Scott Gordon, for IT support; Dr. Gonçalo Abecasis, for advice on the analysis; and Dr. Dale Nyholt, for valuable comments on the original manuscript. We particularly acknowledge Professor Lon Cardon's role in the establishment of this project. We also thank two referees for suggestions that helped to improve the manuscript.

Web Resources

The URLs for data presented herein are as follows:

Genetic map with interpolated genetic map positions used for linkage analyses, http://www.qimr.edu.au/davidD/master_map.dat

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/> (for asthma, atopy, *ADAM33*, *PHF11*, *SETDB2*, *DPP10*, *GPRA*, *BHR*, *NOS1*)

SIB-PAIR 0.99.9, <http://www.qimr.edu.au/davidD/sib-pair.html>

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